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Lesions of Testis and Epididymis Associated with Prenatal Diethylstilbestrol Exposure

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Cryptorchidism and retention of Müllerian duct structures occur with high frequency among the male offspring of CD-1 mice treated with 100 μg diethylstilbestrol/kg body weight on days 9 through 16 of pregnancy. Hyperplasia of the rete testis and Müllerian duct structures were found in many of the DES-treated male mice, as was a low but significant number of reproductive tract neoplasms.

This is a brief review of some developmental changes in the male mouse reproductive tract associated with *in utero* exposure to a synthetic estrogen, diethylstilbestrol (DES).

The knowledge that estrogenic compounds affect the early differentiation and development of the male reproductive tract has been evident for about 50 years; however, the full range of morphological changes has begun to emerge only recently. Interest in the subject has been promoted by the finding of rare gynecologic cancers in daughters of women given DES while pregnant, as well as epididymal cysts, cryptorchidism, and testicular neoplasms among the sons of women receiving DES during pregnancy. Because DES is effective when taken orally and is relatively stable, it provides a model for the study of estrogenic compounds in the environment. Background information is provided in several reviews (*1–3*).

Perhaps one of the most important developmental effects of exogenous estrogen on the male mouse reproductive tract is cryptorchidism. Administration of estrogenic substances to pregnant mice or to neonatal mice has become a frequently used model to study cryptorchidism (*4*). Cryptorchidism is not an insignificant problem among men; it is one of the most frequent indications for pediatric surgery, and in some studies, 2 to 3% of full-term male infants have been found to have retained testes. It is even more frequent among premature male infants (*4*).

The results summarized here have been derived from the study of offspring of CD-1 mice treated with 100 μg DES/kg body weight on days 9 through 16 of pregnancy (*5–9*).

We have chosen not to deal at length with the subtle hypoplastic and degenerative changes associated with cryptorchidism; the well-known testicular changes include incomplete maturation of spermatozoa, atrophy of the seminiferous tubules with thickening of basement membranes, and interstitial fibrosis. Some intra-abdominal testes were infarcted, perhaps due to torsion, and others were reduced to fibrotic nodules with few recognizable features. In a study that involved 277 DES-treated and 122 control mice (10–18 months old), 91% (252/277) of the treated mice had at least one retained testis, 8% had inflammation, and 82% had some degree of degenerative change in the testis. The degenerative changes were categorized by increasingly severe morphological changes which are as follows: (a) relatively normal appearing testes that were smaller than controls, (b) testes with substantially reduced spermatogenesis, containing giant cells within the lumina of the seminiferous tubules, (c) testes composed of atrophied seminiferous tubules with hyalanized basement membranes and thickened arterioles, and (d) testes composed mostly of necrotic or scar tissue with the absence of spermatogenesis. The 82% incidence of degenerative changes in male mice includes those from categories b, c, and d.

In addition to retained testes and degenerative changes, two (1%) of the male mice exposed *in utero* to DES had interstitial cell tumors, and five (2%) had interstitial cell carcinomas of the testis. None of these changes were found among control animals in this study (*9*).

Some strains of laboratory animals, most notably the Fischer 344 rat, have a high prevalence of interstitial cell tumors, but these tumors rarely, if ever, metas-

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Table 1. Rete testis lesions in male mice exposed prenatally to diethylstilbestrol (*6*).[a]

Lesion	Control	DES-100
Hyperplasia	23/96 (24)[b]	130/233 (56)*
Adenocarcinoma	0/96 (0)	11/233 (5)†

[a]Males were the 10- to 18-month-old offspring of CD-1 mice treated with DES (100 μg/kg body weight, injected SC on days 9–16 of gestation). As there was not a statistically significant difference in prevalence of lesions with age, rete testis hyperplasia and lesions resembling adenocarcinoma in each age group have been combined.
[b]Numbers in parentheses are percentages.
*Statistical significance of DES-exposed animals to corresponding age-matched controls by the Fisher exact test, $p < 0.001$.
†$p < 0.05$.

Table 2. Abnormalities in male mice exposed prenatally to diethylstilbestrol (*8*).[a]

Abnormality	Number of animals
Müllerian remnants[b]	
Prominent structure	268/277 (97)[c]*
Cystic structure	121/277 (44)*
Cystic hyperplasia	18/277 (6)*
Hyperplasia	85/277 (31)*
Inflammation	27/277 (10)*
Tumor[d]	23/277 (8)*
Wolffian derivatives[e]	
Cystic epididymis	32/277 (12)*
Inflammation	87/277 (29)*
Sperm granuloma of epididymis	16/277 (6)*
Hyperplasia of epididymal duct	1/277 (0)
Tumor[f]	1/277 (0)

[a]Males were the 10- to 18-month-old offspring of CD-1 mice treated SC with DES (100 μg/kg) on days 9-16 of gestation.
[b]Squamous metaplasia and gland function in male Müllerian remnants are not included in the table because of low incidence.
[c]Numbers in parentheses are percentages.
[d]Tumors of Müllerian remnants are described in Table 3.
[e]At 18 months of age, the control animals had minor inflammatory changes in Wolffian derivatives. One 18-month-old control animal had severe inflammation of the epididymis and another animal had a sperm granuloma. No other lesions were observed in the control 122 animals at any age in this study.
[f]Tumor in the Wolffian derivatives was adenoma of the epididymis.
*Statistically significant difference between DES-exposed animals and corresponding controls; Fisher exact test $p < 0.05$.

Table 3. Tumors in Müllerian remnants in male mice exposed prenatally to diethylstilbestrol.[a]

Lesion	Number
Adenoma	2
Cystadenoma	15
Carcinoma	1
Stromal tumor	2
Stromal sarcoma	1
Complex stromal sarcoma	1
Adenocarcinoma	1

[a] Males were the 10- to 18-month-old offspring of CD-1 mice treated SC with DES (100 μg/kg) body weight on days 9–16 of gestation. The total number of DES-100 males observed in this study was 277.

tasize. In mice, however, some interstitial cell tumors are considered malignant and distant metastases have been reported (*10*). Interstitial cell tumors in control CD-1 mice up to the age of 18 months are quite rare. We have not found these tumors in the control mice in a number of studies. Although the number of neoplasms in DES-treated animals was low, the proportion with features consistent with malignancy (irregular edges, necrosis, hemorrhage, invasion of blood vessels, and extension into the spermatic cord) was high (*9*).

Subgroups of animals (96 control, 233 DES-treated) were identified on the basis of having adequate sections of the rete testis for study. As there was little background information on proliferative lesions of mouse rete testis, a conservative approach was taken in that any piling up of cells in the rete testis of control males was termed "hyperplasia," whereas a more rigorous requirement of a multifocal or diffuse change was placed on DES-treated animals. The results are shown in Table 1.

In addition to the statistically significant increase in hyperplasia in DES-treated animals, neoplasms of the rete testis occurred in 11 of 233 mice (5%). These lesions had the morphological features of human rete testis adenocarcinomas, usually being papillary, but sometimes with tubular or sertoliform appearing areas.

The embryonic origin of the rete testis and Sertoli cells has been a matter of controversy. Byskov reports that the rete testis is probably derived from the mesonephric system (*11*); reviewing her own work and that of others, she concluded that some, if not all, Sertoli cells are also derived from the mesonephros. This would be consistent with our finding of occasional rete testis neoplasms that have some features of Sertoli cell neoplasms (*12*).

One of the more striking findings among DES-treated animals is the persistence of Müllerian structures resembling portions of the mature female reproductive tract. Cystic Müllerian structures with oviduct-type epithelium frequently were found adjacent to the epididymal duct. The range of cystic and proliferative changes seen in these Müllerian remnants, which resembled all portions of oviduct and uterus, are summarized in Table 2.

In normal development of the male reproductive tract, a small portion of the Müllerian duct sometimes persists to form appendix testes and prostatic utricle. However, persistence of the Müllerian duct is a usual occurrence in CD-1 mice that received DES *in utero;* the fimbria of the oviduct in these animals is larger than the appendix testes of control animals, and the homologues of the oviduct and uterus in males may occasionally exceed the size of the normal structures in female mice. Pathological changes may develop in these male homologues of the oviduct and uterus (*8*). These benign and malignant proliferative lesions are summarized in Table 3.

A substance produced by Sertoli cells, called Müllerian inhibiting substance (MIS) (*13*) or anti-Müllerian hormone (*14*), has an important influence on regression of the Müllerian duct in males. The whole concept of Müllerian duct regression is a subject of vigorous investigation, and the words "regression" and "persistence" are probably oversimplifications. In heterotypic

organ culture, testes from DES-treated male fetuses cocultured with Müllerian ducts from normal animals were capable of producing regression, but Müllerian ducts from DES-treated animals cocultured with normal testes did not regress (*15*). This suggests a defect in the ability of the DES-exposed reproductive tract tissue to respond to MIS. A persistent Müllerian duct syndrome has been described in human males, usually associated with cryptorchidism and Leydig cell hyperplasia. These men may have uteri, oviducts, and the upper one-third of the vagina (*16*).

In summary, the occurrence of several types of rare neoplasms in the male offspring of mice that received DES during pregnancy suggests that although the neoplasms are expressed later in life, interference with normal development at an early stage may be necessary for their development.

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